TRANSFER RNA: A COMPARISON BY GEL ELECTROPHORESIS OF THE tRNA IN HeLa CYTOPLASM, HeLa MITOCHONDRIAL FRACTION, AND E. COLI

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Communicated by Irwin C. Gunsalus, June 11, 1969

Abstract.—A class of transfer RNA separable from the majority of transfer RNA by gel electrophoresis has been observed in *E. coli* and in the mitochondrial fraction of HeLa cells but not in HeLa cytoplasm. The size of this class of transfer RNA, as judged by its mobility on polyacrylamide gel, is intermediate between the major part of the transfer RNA and 5*S* RNA. It exists in part associated with the ribosomes, and its amino acid acceptor activity is different from the major class of transfer RNA.

Recent reports have indicated that some of the RNA species associated with the mitochondria are unique to that organelle.[1–7] In this report the separation by gel electrophoresis of the transfer RNA (tRNA) in the HeLa cell mitochondrial fraction and in *E. coli* into major and minor classes is described. The major class of tRNA has been designated *A* and the minor class *B*. A more complete name of tRNA *B* must await further description.

Materials and Methods.—Cell growth: HeLa cells were grown in suspension culture as described by Eagle[8] to a concentration of 2–5 $\times$ 10^5 cells/ml. *E. coli* strain Hfr 3000 was grown in modified TPA medium[9] and an extract prepared by grinding the cells with alumina.[10]

Preparation of HeLa mitochondria: HeLa cells were suspended in 10 mM Tris-Cl, pH 7.4, containing 0.1 mM EDTA and 0.25 *M* sucrose, and were broken by being sheared in a metal ball homogenizer. Nuclei were removed by centrifugation at 3,000 rpm for 3 min in an angle rotor, the supernatant was made 10 mM in EDTA, and a crude mitochondrial pellet collected by centrifugation at 9,000 rpm for 10 min to yield as supernatant a mitochondria-free cytoplasm. The pellet was resuspended in 10 mM Tris-Cl, pH 7.4, 10 mM EDTA, 0.25 *M* sucrose, and subjected to centrifugation at 2,500 rpm for 2 min to remove remaining nuclei, and the mitochondria were collected by centrifugation at 9,000 rpm for 10 min. The pellet was resuspended in Tris-EDTA-sucrose, layered on a 30-ml sucrose gradient (25% w/w to 55% w/w in 10 mM Tris-Cl, pH 7.4, 0.1 mM EDTA) and the mitochondria were banded by centrifugation for 2 hr at 24,000 rpm, 4°, in a Spinco SW25.1 rotor. The concentration of mitochondria was measured spectrophotometrically by assaying for cytochrome oxidase.[11] The major band was found at density 1.18.

Preparation of ribosomes: Ribosomes were prepared from the HeLa mitochondrial fraction (isopycnic gradient) by collecting the mitochondria by centrifugation as described above, then lysing the mitochondrial membrane with 0.5% deoxycholate. Ribosomes from both the mitochondrial fraction and the crude extract of *E. coli* were collected by centrifugation for 2.5 hr at 49,000 rpm, 4°C, in the Spinco Ti50 rotor. The RNA in the pellet and in the supernatant was purified as described below.

Preparation and analysis of RNA: RNA was extracted at room temperature and 60° with sodium dodecyl sulfate and phenol as previously described.[12]

The low-molecular-weight RNA was analyzed by electrophoresis on 10% polyacrylamide gel columns 18 and 30 cm long as described by Loening.[13] The gel columns were

fractionated by being cut into 1-mm slices. Each gel slice was allowed to stand for 16 hr at room temperature in 0.5 ml of 1 M NH_3 to elute the radioactive RNA. Radioactivity was determined in a liquid scintillation counter after the addition of 10 ml of Bray's[14] solution to each gel-NH_3 mixture. Intact, biologically active RNA was eluted from the gel slices by suspending each slice in 2 ml of the electrode buffer[13] and shaking for 16 hr at 30°. The 30-cm gel columns gave a complete separation of tRNA A and B.

Aminoacyl tRNA synthetases were prepared and the amino acid acceptor activity of the tRNA was assayed according to Muench and Berg.[15]

Results.—A mitochondrial pellet containing cytochrome oxidase activity can be isolated from HeLa cell cytoplasm. This activity can be banded in a sucrose density gradient at $\rho = 1.18$. This density is in agreement with the reported values for mammalian mitochondria.[16]

The low-molecular-weight RNA associated with HeLa mitochondria was studied by labeling the RNA of a cell culture with H^3-uridine for two generations (48 hr) and extracting the RNA from the mitochondrial fraction of the isopycnic gradient and the corresponding cytoplasm (minus mitochondria). The low-molecular-weight RNA in both fractions was analyzed by electrophoresis on 10 per cent polyacrylamide gel. The electrophoretic profile of the low-molecular-weight RNA in the HeLa mitochondrial fraction and in HeLa cytoplasm is shown in Figure 1. Both fractions contain 4S and 5S RNA. The mitochondrial fraction (Fig. 1a), however, contains an additional peak (designated by an arrow) with an electrophoretic mobility between that of 4S (tRNA) and 5S RNA which is not observed in the cytoplasm (Fig. 1b). This species of RNA has been consistently observed in all preparations of HeLa mitochondria. It should be noted that the RNA in both fractions is prepared by identical procedures. Figure 1c shows the low-molecular-weight RNA of *E. coli* labeled with P^{32}. A compo-

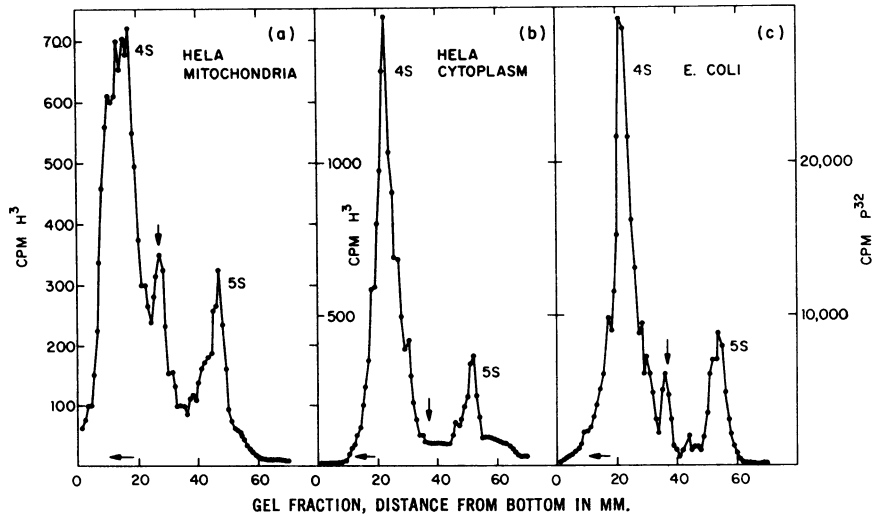

FIG. 1.—Gel electrophoresis of low-molecular-weight RNA from HeLa mitochondrial fraction, HeLa cytoplasm, and *E. coli*. HeLa cells in culture were labeled with H^3-uridine, and *E. coli* with P^{32}, for two generations. RNA, prepared as described in *Methods*, was analyzed on an 18-cm, 10% gel; 6 ma/gel, 8.5 hr. The lower 6.5 cm of each gel is shown.

nent(s) with a mobility similar to that in the HeLa mitochondrial fraction is also evident (*arrow*). The same RNA profile is observed when *E. coli* RNA is labeled with C^{14} or H^3-uracil. In this report the broad peak of 4*S* RNA (Fig. 1) will be referred to as tRNA *A*, and the peak (*arrow*) intermediate between the 4*S* and 5*S* RNA in the HeLa mitochondrial fraction and *E. coli* will be referred to as tRNA *B*, for reasons stated later in this paper.

The material under the peak (*arrow*) with a mobility between 4*S* and 5*S* RNA has characteristics of RNA: (1) It is completely acid-soluble after 16 hours in 0.3 *M* KOH, and (2) its ultraviolet spectrum is similar (maximum at 259 mμ) to that of RNA.

The tRNA *B* is not an artifact of isolation since there is no qualitative or quantitative change in the gel-electrophoretic profile when the low-molecular-weight RNA of the HeLa mitochondrial fraction and of *E. coli* is heated (60°) in 1 *M* NaCl or 0.01 *M* NaCl.[17] The possibility that the intermediate peak is a dimer of 4*S* RNA[18] can be excluded, since a dimer would be expected to have a mobility less than 5*S* RNA and would be unstable in the concentration of EDTA used for preparing the RNA (10 mM) and for performing the gel electrophoresis (5 mM). A further argument against a dimer is the absence of the tRNA *B* in the cytoplasm.

In Figure 2 the mobility of the tRNA *B* (*arrow*) from the HeLa mitochondrial fraction is compared to that from *E. coli*. The tRNA *B* from both sources has similar mobilities, indicating similar sizes and configurations. Further studies, however, will be necessary to determine if the tRNA *B* from both sources has other similar properties.

In order to determine the cellular localization of the tRNA *B*, a ribosomal

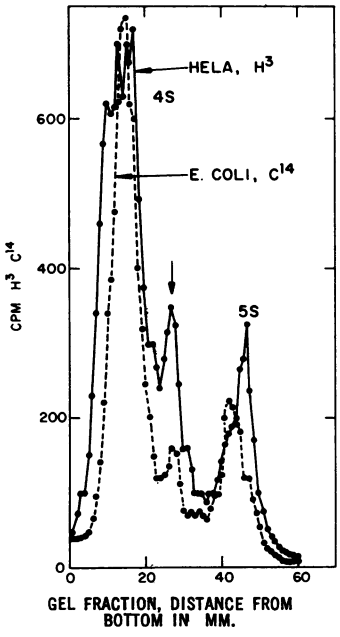

FIG. 2.—Coelectrophoresis of *E. coli* and HeLa mitochondrial tRNA *A* and *B*. H^3-RNA from the HeLa mitochondrial fraction and C^{14}-RNA of *E. coli* were mixed and analyzed on an 18-cm, 10% gel; 6 ma/gel, 8.5 hr. The lower 6 cm of the gel is shown.

pellet and a ribosome-free supernatant were prepared from the HeLa mitochondrial fraction as described in *Methods*. The low-molecular-weight RNA in each fraction is shown in Figure 3. Like tRNA *A*, part of the tRNA *B* is associated with the ribosomes (Fig. 3*a*) and part is free (Fig. 3*b*). An identical experiment performed with *E. coli* gave similar results (Fig. 3*c* and *d*). In both systems a majority of the tRNA *B*, like tRNA *A*, is not associated with the ribosomes. This suggests that the tRNA *B* may have a biological role similar to the tRNA *A* but different in size and/or configuration.

The tRNA *A* and tRNA *B* of *E. coli* were separated on a 30-cm, 10 per cent polyacrylamide gel column and assayed for amino acid acceptor activity. Since the amount of tRNA *B* prepared was very limited, acceptor activity was not tested with each individual amino acid; instead, each class of RNA was assayed with 15 amino acids in three groups of five amino acids each. The results of this experiment are shown in Table 1. Two conclusions can be drawn from this experiment: (1) The tRNA *B* of *E. coli* can be charged with amino acids, and (2) the activities of the tRNA *A* and tRNA *B* are different toward the three

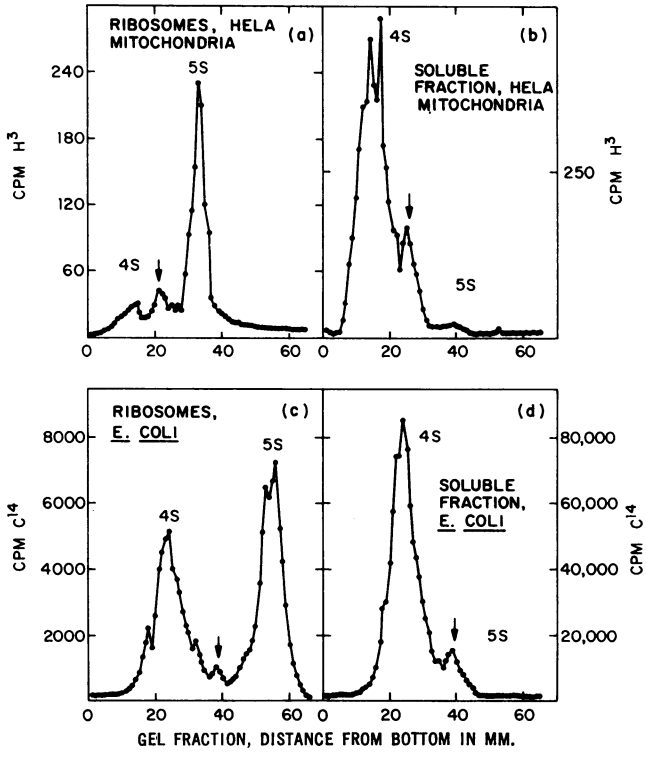

FIG. 3.—tRNA *A* and *B* in association with and free of the ribosomes in the HeLa mitochondrial fraction and in *E. coli*. The RNA of HeLa cells and *E. coli* was labeled with H^3-uridine and C^{14}-uridine, respectively, as described in Fig. 1. A ribosomal pellet and a ribosome-free soluble fraction were prepared and the RNA was extracted as described in *Methods*.

groups of amino acids. Although it has not yet been proved, we presume that the tRNA *B* in the HeLa mitochondrial fraction also has amino acid acceptor activity.

Discussion.—From the data in this report, three conclusions can be drawn: (1) The HeLa mitochondrial fraction has a class of low-molecular-weight RNA (tRNA *B*) that is not found in HeLa cytoplasm, (2) *E. coli* contains a class of RNA that is similar in size to that in the HeLa mitochondrial fraction, and (3) this class of RNA in *E. coli* has a general biological activity like that of transfer RNA.

The observation of the tRNA *B* by gel electrophoresis is reproducible and is not an artifact of isolation. The occurrence of this RNA in the HeLa mitochondrial fraction is not the result of bacterial contamination, for the HeLa cultures used in these experiments were shown to be free of mycoplasma.

TABLE 1. *Amino acid acceptor activity of E. coli tRNA A and B.*

	Amino Acid Mixture (cpm)		
RNA	I	II	III
A	1681	868	931
B	438	1287	1975

The assay mixture contained 15 μg of either *E. coli* tRNA *A* or *B*, 100 mM Tris-HCl (pH 7.2), 10 mM $MgCl_2$, 1 mM ATP, 4 mM glutathione, 10 mM KCl, 50 μM each of C^{14} AA (10 μc/μmole), and a saturating level of *E. coli* activating enzymes in a total volume of 0.5 ml. The assay mixture was incubated 15 min at 37°C and the C^{14} AA–RNA precipitated with cold 5% TCA. The precipitate was collected on a Millipore filter, washed, and the radioactivity counted in a liquid scintillation counter. Amino acid mixture I contained alanine, arginine, aspartic acid, glutamic acid, and glycine; mixture II contained histidine, isoleucine, leucine, lysine, and phenylalanine; mixture III contained proline, serine, threonine, tyrosine, and valine.

Since tRNA *B* of HeLa cells is localized in the mitochondrial fraction, is its site of synthesis a mitochondrial DNA template or a nuclear DNA template? RNA-DNA hybridization studies in progress should resolve this question.

We have shown that the tRNA *B* of *E. coli* can be charged with amino acids. Its amino acid specificity, however, is different from that of the major class of tRNA (tRNA *A*) in *E. coli*. This may simply reflect the existence of a small part of the total tRNA having a larger molecular weight and/or different configuration. Further study may, however, reveal different functional roles for tRNA *A* and *B*.

N-Formylmethionyl tRNA has been found in the mitochondrial fraction of rat liver[19] and HeLa cells[20] but not in the corresponding cytoplasms. Transfer RNA *B* reported here is not only *N*-formylmethionyl tRNA since (1) the amount of tRNA *B* compared with the amount of tRNA *A* (*ca.* 5%) is too large in both *E. coli* and the HeLa mitochondrial fraction, and (2) tRNA *B* of *E. coli* accepts amino acids other than methionine (Table 1).

Although not rigorously proved, protein synthesis in the mitochondrion of mammalian cells has been suggested to have some characteristics similar to protein synthesis in bacteria rather than in the mammalian cytoplasm. Our findings also suggest that the low-molecular-weight RNA of the HeLa mitochondrial fraction may have features which more closely resemble the RNA in *E. coli* than that in HeLa cytoplasm.

The authors thank Miss Diana Fahey for excellent technical assistance throughout the course of this research.

* Contribution no. 1572.

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